TABLE II

NaKATPase transporting capacity in the rectal gland

Assay conditions			NaKATPase activity	Transport capacity* Na+ efflux = Cl^- secretion	
T °C	Na	K(mM)	µM Pi/mg of protein/hr	µEq/hr/g	
				a	b
37	100	20	38.1 ± 1.9(7)	12000	6000
37	50	5	21.0 ± 1.3(4)	6800	3400
20	100	20	6	1800	900
20	50	5	3?	900	450

*Calculated values based on: 1) 108 ± 3(28) mg of protein per gram of rectal gland
2) a. 3 Na removed per ATP hydrolyzed (red cells)
b. 1.5 Na removed per ATP hydrolyzed (rectal gland vesicles)

Average Cl^- secretion in the rectal gland = 1440 ± 41(250)

more Cl^- per Na+, clearly an electrogenic mechanism. If such is not the case, additional chloride carriers or pump have to be postulated.

EFFECT OF VASOACTIVE INTESTINAL PEPTIDE, SOMATOSTATIN AND THEOPHYLLINE ON ACTIVE CHLORIDE TRANSPORT AND CYCLIC AMP METABOLISM IN THE RECTAL GLAND OF *Squalus acanthias*

Jeffrey S. Stoff, Robert Rosa, Ralph Hallac, Diane Leone, Patricio Silva and Franklin H. Epstein
Dept. of Medicine and Thorndike Laboratory of Harvard Medical School at Beth Israel Hospital, Boston, Massachusetts

The rectal gland of the dogfish shark secretes a hypertonic solution of sodium chloride by a process involving the active transport of chloride. This gland is composed of a homogenous population of branching secretory tubules. These tubules are formed by a secretory type of epithelium with rich basal-lateral infoldings and a specialized apical surface demonstrating numerous microvilli (Doyle, Bulletin 15:28-30, 1975). The gland has a single artery, vein and duct and can therefore, easily be removed from the shark and perfused at seawater temperature in the laboratory with artificial shark-Ringers solution as previously described (Stoff et al., J. Exptl. Zool. 199:443-448, 1977). Studies from our laboratory have revealed a number of key characters of rectal gland secretion. (1) The duct fluid contains sodium chloride at 1.5 - 2.0 times the concentration of the shark plasma or artificial perfusate. This is approximately the concentration of sodium chloride in seawater. (2) The duct secretion is isosmotic with plasma by virtue of the fact that it contains little or no urea. (2) Measurement of the electrochemical driving forces across the contraluminal membranes indicate that chloride is the actively transported ion since the electrical potential of the duct is negative to the perfusate. (4) The entry step for chloride is tightly coupled to sodium and probably involves a Na-Cl carrier localized to the contraluminal membrane. (5) This requirement of sodium for chloride entry is further exemplified by the dependence of chloride transport on Na-K-ATPase activity. In these respects, active chloride transport in the rectal gland is similar to that in other electrolyte transporting epithelia like amphibian skin and urinary bladder; mammalian cornea and intestine; and the thick ascending limb of the loop of Henle. Recently we have demonstrated that secretion by the rectal gland was markedly stimulated by theophylline and cyclic AMP (Stoff et al., ibid. 199:443-448, 1977). The responses to theophylline and dibutyryl cyclic AMP are quite prompt and secretion increases by 10- to

30-fold. There is an increase in the concentration of sodium chloride in the duct fluid as well as in the secretion volume, and the electrical potential difference of the duct becomes more negative. This response to theophylline is dose dependent with increasing concentrations of theophylline eliciting an increased secretory response. In addition, this response to theophylline is associated with a parallel rise in the intracellular level of cyclic AMP level (Stoff et al., Bulletin 16:95-98, 1976).

Thus, we had determined that cyclic AMP (second messenger) is the intracellular mediator of active chloride transport in the rectal gland. Since this association between cyclic AMP and electrolyte transport usually implies hormonal control (first messenger), we explored a number of hormones for their possible effects on chloride transport and cyclic AMP metabolism. No effect was found for a large number of peptide hormones and neurohumoral factors these included; vasopressin, several oxytocin derivatives normally found in the posterior lobe of the pituitary of the dogfish, norepinephrine, epinephrine, serotonin, substance P and calcitonin. Since the rectal gland is an appendage of the elasmobranch hind gut, a number of hormones which stimulate intestinal secretion were studied as well; these include pentagastrin and a structurally related group of peptide hormones, secretin, glucagon and vasoactive intestinal peptide or VIP. (GIP or gastrin inhibitory peptide has not been studied at this time.) All these hormones have extensive regions of structural homology. However, only VIP produced an increase in the chloride secretory response. As with theophylline, VIP stimulation resulted in an increase in the volume and concentration of sodium chloride in the duct fluid as well as an increase in electronegativity of the lumen (Stoff et al., Bulletin 17:66-69, 1977). This response is dose related, over a concentration range of 10^{-8} - 10^{-6} M (Figure 1). Furthermore, this effect of VIP on the volume of rectal gland secretion and the rate of chloride transport is potentiated by theophylline (Figure 2). A submaximal concentration of theophylline or VIP produced a small increase in

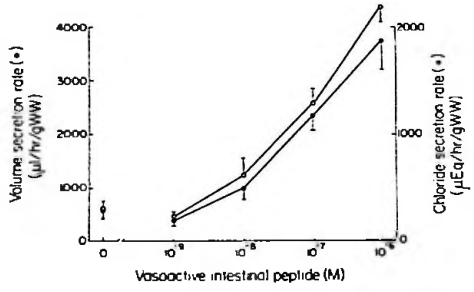

Figure 1. Dose response of vasoactive intestinal peptide on volume and chloride secretion rate in the isolated perfused rectal gland. Each value represents mean ± SEM. Sample size varies between 4 and 15 at each concentration studied.

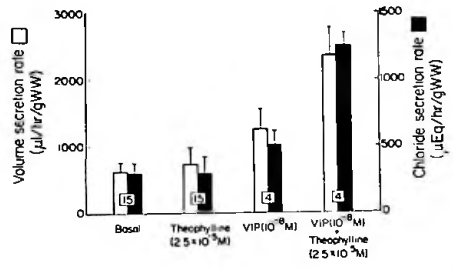

Figure 2. Effect of VIP and theophylline on volume and chloride secretion rate in the isolated perfused rectal gland. Each value represents mean ± SEM. Sample size is indicated at base of each bar. Both VIP and theophylline increase secretory rate and have a synergistic effect when present together.

volume and chloride secretion while a synergistic response resulted with the combined addition of the two agents. This increase in chloride transport produced by VIP is associated with an increase in intracellular cyclic AMP content (Figure 3). This concentration of theophylline (10^{-3} M) resulted in a rise in intracellular cyclic AMP. VIP (10^{-8} M) alone produced a small increase in cyclic AMP which is potentiated by theophylline. A similar pattern of response was seen at 10^{-6} M VIP. In contrast, secretin alone produced no increase in cyclic AMP while the addition of theophylline produced an increase comparable to that seen with theophylline alone.

Recent studies on mammalian intestinal secretion indicate that somatostatin, a tetradecapeptide which was first isolated from the hypothalmus and found to inhibit growth hormone release, also inhibited VIP induced intestinal secretion (Carter et al., Gastroenterology 74:726-730, 1978). We

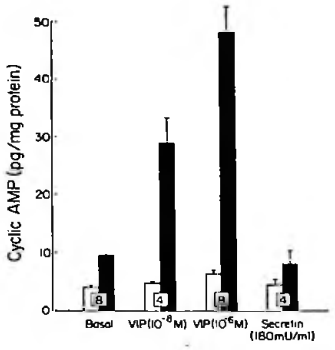

Figure 3. Effect of VIP and theophylline on intracellular cyclic AMP level in rectal gland slices. Cyclic AMP was measured by protein kinase binding assay (Stoff et al., PNAS 69:805-808, 1972). VIP increases intracellular cyclic AMP content at each concentration tested and the addition of theophylline results in a synergistic effect. Secretin has no effect. All values are means ± SEM.

studied the effect of somatostatin on the VIP response in the rectal gland (Figure 4). In these studies, VIP (10^{-6} M) increased both the volume and chloride secretion rate while somatostatin (1.4×10^{-7} M) alone had no effect, but completely blocked the VIP induced response. In other studies, not shown here we found that this effect of somatostatin was reversible. Furthermore, somatostatin had little or no effect on theophylline or cyclic AMP induced secretory response.

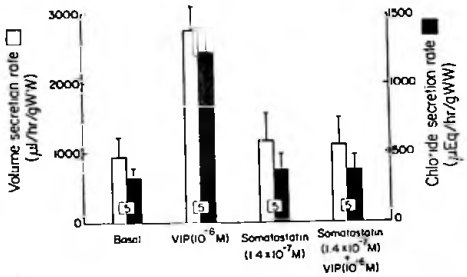

Figure 4. Effect of VIP and somatostatin on volume and chloride secretion rate in the isolated perfused rectal gland. Somatostatin has no effect alone but inhibits the secretory response to VIP. All values are means ± SEM.

These studies demonstrate that vasoactive intestinal peptide stimulates active chloride transport by a mechanism mediated by cyclic AMP. This response is prompt, dose related and reversibly blocked by somatostatin. Since somatostatin completely inhibited the VIP-induced secretory response but had little effect on theophylline or cyclic AMP-induced secretion it seems likely that somatostatin exerts its effect by preventing the VIP induced increase in cyclic AMP. Recent studies indicate that both VIP and somatostatin are present in the plasma and gastrointestinal tract of these cartilagenous fish as well as in mammals (Falkmer et al., Metabolism 27 (Suppl. 1):1196, 1978). The precise role of these peptides in the regulation of chloride transport in the rectal gland remains to be established.

FIELD STUDIES OF CRUDE OIL TOXICITY IN SEABIRDS

Ronald G. Butler, Peter Lukasiewicz, Wayne Trivelpiece and William B. Kinter, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

Recent investigations indicate that sublethal doses of crude oil can result in decreased weight gain, hypertrophy of adrenal, nasal and hepatic tissue, decreased efficiency of intestinal transport, and decreased osmoregulatory ability in young seabirds raised under controlled laboratory conditions (Miller et al., Science 199:315-317, 1978). It is clear that such studies must be extended to wild populations to facilitate our understanding of the biological impact of environmental contamination on marine avifauna. To this end, a field team established a camp on Little Duck Island, Maine, in